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Research Article

Detection of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* in Ready-to-Eat Food in Al-Ahsa Province, Saudi Arabia

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ABSTRACT

The study aimed to identify and characterize foodborne- *Staphylococcus aureus, Escherichia coli* and Salmonella spp. in Al-Ahsa Province, Kingdom of Saudi Arabia (KSA), as potential reservoir of human infection and transmitters of antimicrobial resistance. A total of 90 sandwich samples (consist of minced meat and vegetables) were aseptically collected from fast-food cafeterias. Conventional bacteriological techniques were used to isolate *Staph. aureus*, *E. coli* and *Salmonella spp.* For *E. coli* isolates, molecular analysis was made. *Staph. aureus* was confirmed from 11.11% of specimens of which 30% were MRSA. MRSA were resistant to erythromycin, nitrofurantoin and Trimethoprim/ Sulfamethoxazole. Non-MRSA were resistant to ciprofloxacin and all *Staph. aureus* isolates were sensitive to vancomycin which may be a choice for treatment. At a rate of 5.56%, *E. coli* was confirmed by conventional techniques and VITEK 2 system; *E. coli* strain O157: H7 was not identified from the isolates. Molecular analysis indicated that 4 strains belonged to Shiga toxigenic *E. coli* (STEC) family and one strain was *Shigella flexneri.* Antimicrobial susceptibility analysis of isolates showed two strains (40%) were extended-spectrum β-lactamases (ESBL) positive that were demonstrated to be susceptible to imipenem but resistant to ciprofloxacin. On the other hand, three strains (60%) were identified as ESBL negative which were susceptible to all tested antibiotics. *Salmonella* spp. were not isolated from any food specimen in the present study. From ready-to-eat food in Al-Ahsa Province, KSA, *Staph. aureus* MRSA and Non-MRSA were susceptible to vancomycin. Using molecular methods, *E. coli* and *Shig. flexneri* were confirmed from food with ESBL positive susceptible to imipenem but resistant to ciprofloxacin.

Keywords: Fast-food; Meat; *Staph. aureus*; MRSA; *E. coli*; ESBL; Antibiotics; Saudi Arabia

INTRODUCTION

Meat is a highly nutritious item of food. However, it has been well-known as a potential medium for spreading food-borne diseases. This is due to its high water activity, high protein content, and approximately neutral pH, which create favourable conditions for the multiplication of bacteria [1]. Food-borne diseases (FBD) caused by bacterial species prevail at unacceptably high frequencies in industrialized and developing countries as well [2].

Staphylococcus is a spherical, coccal, nonsporulating non-motile bacterium that appears microscopically in short chains or grape-like clusters.

Staphylococcal food poisoning (SFP) is one of the most common FBD and is of major concern in public health programs worldwide [3-5]. It occurs following the ingestion of staphylococcal enterotoxins (SEs) that are produced by enterotoxigenic strains of *Staph. aureus* [6,7]. The first description of staphylococcal food-borne disease was investigated in Michigan (USA) in 1884 by Vaughan and Sternberg [5]. If food is prepared in a central location and widely distributed, SFP outbreaks can have grave consequences impacting thousands of people. For example, over 13,000 cases of SFP occurred in Japan in 2000 as a result of contamination of milk at a dairy-food-production plant [8,9]. Another study [10] detected *Staph. aureus* in ready-to-eat food products prepared at a large processing plant in West Indies. The overall occurrence of *Staph. aureus* in cattle slaughterhouses in South India was 50.8% [11]. Methicillin Resistant *Staph. aureus* (MRSA) is a type of *Staph. aureus* which gained resistance to common antibiotic such as methicillin which is a semisynthetic penicillin.

Escherichia coli of Family Enterobacteriaceae is commonly found as normal flora in the gut of humans and warm-blooded animals. Some strains such as entero-haemorrhagic *E. coli* (EHEC) and Shiga toxin-producing *E. coli* (STEC), can cause severe food-borne disease. It is transmitted to humans primarily through

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consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts [12]. Its significance as a public health problem was recognized in 1982, following an outbreak associated with consumption of undercooked ground beef in the United States [13,14]. STEC produces toxins, known as Shiga-like toxins because of their similarity to the toxins produced by *Shigella dysenteriae*. STEC species are classified into 2 subtypes: O157 and non–O157. Non-O157 sero-types are the most prevalent microorganisms in reported foodborne outbreaks in the United States and other countries [15]. Food poisoning outbreaks linked to contaminated sprouts in which *Salmonella spp.* or *E coli* 0157:H7 was identified as the causative pathogen, were documented [16,17].

A study in the KSA, investigated presence of *E. coli* in meat samples collected from different abattoirs located in Riyadh area. *E. coli* strains were recovered in a percentage of 11.33%; sero-typing of *E. coli* isolates revealed, 6 (4%) strains O157: H7, 5 (3.33%) strains O111 and 4 (2.67%) strains O174: H2 and only two (1.33%) strains were identified as O22: H8. Shiga toxin2 was detected in 94.12% serotypes of *E. coli* [18]. Further *E. coli* and *Salmonella spp.* were screened from meat samples obtained from large hypermarkets, groceries and small butcher shops in Jeddah, KSA. Rate of *E. coli* recovery was 20% in hypermarkets, 40% in groceries and 65% in small butcher shops. *Salmonella spp.* were detected at percentages of 5% in hypermarkets, 25% in groceries and 45% in small butcher shops [19]. An outbreak of food poising occurred in Al-Hofuf in 2009 associated with eating chicken shawarma contaminated with *Salmonella enteritidis* from a restaurant [20]. Surveillance of food borne outbreak was done in Qassim area in 2006, where the results obtained showed that 64.5% of both male and female suffered from gastroenteritis. The main causative agent was *Salmonella spp.* then *Staph. aureus* [21].

Extended-spectrum β-lactamases (ESBLs) are a large group of plasmid-mediated enzymes [22,23] which induce resistance to most beta-lactam antibiotics. Gram-negative bacterial species that produce ESBLs have been identified in city rivers, sewage [24], livestock [25], companion animals [26] and meat obtained from supermarkets [27].

Traditionally, identification of microorganisms has relied on conventional methods which cannot fully identify bacterial isolates. The VITEK 2 Automated System (bioMérieux) is one of the most widely used instruments in clinical microbiology laboratories for the identification and evaluation of anti-microbial susceptibility profile of bacteria including the detection of ESBLs produced by *E. coli* [28].

Clinical microbiology laboratories are increasingly relying on partial 16S rRNA gene sequencing for bacterial identification [29,30]. Many workers have demonstrated improved accuracy with 16S rRNA gene sequencing using GenBank databases [29-31].

Thorough search of the literature showed that there were no reports of foodborne pathogenic bacteria in Al-Ahsa Province, Eastern Region, KSA. Therefore, the objectives of the present study were to identify and characterize foodborne- *Staph. aureus, E. coli* and *Salmonella spp.* in the study area, as potential reservoir of human infection and transmitters of antimicrobial resistance.

MATERIALS AND METHODS

Study area

The study was conducted in Al-Ahsa Province, Eastern Region, KSA. Microbiological investigation was done in the Bacteriology Laboratory, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, King Faisal University.

Sample collection

Sandwich samples (consist of minced meat and vegetables) were purchased from fast-food cafeterias in Al-Ahsa Province. With the aid of a province map, the area was divided into North, South, East, West and Central parts. From each part, 18 samples were collected. A total of 90 samples were aseptically collected in the same way delivered to the consumer. All samples were collected during breakfast time (between 8 to 11 AM), put into sterile plastic bags and brought to the laboratory in an ice box within 2 h from the time of purchase. In the laboratory samples were stored at 4°C and microbiological analysis started immediately after reception of samples. Bacterial isolation was done according to [32] with some modifications, a 25-g portion was weighed aseptically in sterile stomacher bags (Seward Medical StomacherR Bags), diluted with 225 ml of sterilized 0.1% w/v peptone water (Oxoid) and macerated in a stomacher for 3 min.

Microbiological analysis

A. **Staphylococci:** Triplicate swabs were prepared from each specimen and cultured on Baird Parker agar (BD Diagnostics, Franklin Lakes, NJ, USA), supplemented with egg yolk tellurite emulsion. The plates were incubated at 37°C for 18–24 h and recovered single colonies were streaked onto 5% citrated sheep blood agar plates and incubated at 37°C overnight. Cultural characteristics, morphology and biochemical tests were done to identify staphylococci. Confirmation of the identification of isolates and antimicrobial susceptibility profile to a wide range of antimicrobial drugs were done by VITEK 2 technique (bioMerieux, France). (Sensitivity and resistance in VITEK 2 technique are calculated from minimal inhibition concentration "MIC" i.e. minimum concentration of the antibiotic that inhibits growth of the tested species).

B. **Enterobacteriaceae:** Bacteriological analyses were done according to the protocol of American Public Health Association [33]. Triplicate swabs were prepared from each specimen and cultured on Columbia agar (Oxoid, UK) containing 5% citrated sheep blood and MacConkey agar (Oxoid). The plates were incubated aerobically and anaerobically for 18 to 24 h at 37°C and for a further 24 h if bacterial growth had not ensued. Bacteriological common diagnostic procedures as described by Koneman et al., [34] including cultural characteristics, Gram staining and bio-chemical confirmation were used for culture identification. Hekton enteric agar was used as a differential medium for members of family Enterobacteriaceae. Sorbitol MacConkey agar (SMA), (Oxoid) was used to type *E. coli* O157 from the obtained isolates. For Salmonella spp., pre-enrichment in

selenite broth (Oxoid) then plating on differential media as describe above.

Confirmation of the identification of isolates (Table 1) and antimicrobial susceptibility profile to a wide range of antimicrobial drugs (Table 2) were done by VITEK 2 technique (bioMerieux, France).

Molecular analysis

Individual isolates of *E. coli* were frozen at −70°C in brain heart infusion broth containing 15% glycerol for further use. Molecular investigation was carried out as described by Jackson [35] with some modification. Each isolate was sub-cultured into Luria Bertani broth and incubated at 37°C for 18 hours. A volume of 1 ml was centrifuged at 3500 g/ 3 min in micro-centrifuge tubes and the supernatant (S/N) was discarded. The precipitate was suspended in 200 µl of extraction buffer (0.1M Tris-HCl pH 7.5, 0.05M EDTA pH 8.0, 1.25% SDS) and mixed well. The tube was incubated at 63°C for 3 min, brought to room temperature, washed at 3500 g/ 3 min and S/N was removed gently to a new tube. An equal volume of absolute ethanol was added to precipitate DNA, washed for 3 min at 3500x g and dried by air flow.

PCR on 16S rRNA using 27F and 1492R primers was performed by Macrogen Inc. (Seoul, South Korea). Sequences of the primers were:

Primer Type Type 2 Sequence (5 to 3) Reference

27F Universal AgAgTTTgATCMTGGCTCAg

1492R Universal TACggYTACCTTgTTACgACTT Zhang et al. [36].

16S ribosomal RNA gene sequence analysis:

Determined sequences were compared with sequences available in GeneBank, EMBL, and DDBJ databases using the BLAST algorithm,15 through the National centre for biotechnology information server (www.ncbi.nlm.nih.gov) and with sequences available in Ribosomal database project (RDP-II) (http://rdp.cme.msu.edu), release 9.59, by use of Sequence match algorithm. 9 In order to assign isolate to a species, each derived sequence aligned by the BLAST algorithm, yielded at least 99% similarity score with identified species in the BLAST search, and the highest S-ab value with identified species in the Sequence match search.

RESULTS

Microbiological analysis

A. Staphylococci: Out of the examined food specimens, *Staph. aureus*

Table 1: Biochemical profile by VITEK 2 system of *E. coli* isolates from ready-to-eat meat sandwiches Al-Ahsa province, KSA. **Key:** 1. Ala-phe-pro-aramidase 2. H2S production 3. β-Glucosidase 4. L-proline Arylamidase 5. Sucrose 6.L-Lactose alkalinisation 7. Glysine Arylamidase 8. 0/129 resistance 9. Adonitol 10. β-N-Acetyl 11. D-Maltose 12. Lipase 13. D-Tagatose 14. α-Glucosidase 15. Ornithine decarboxylase 16. Glu-Gly-Arg-Arylamidase 17. L Pyrrolydonyl-Arylamidase 18. Glutamyl Arylamidase 19. D-Manitol 20. Palatinose 21. D-Trehalose 22. Succinate alkalinisation 23. Lysine decarboxylase 24. L-Malate assimilation 25. L-Arabinose 26. D-Glucose 27. D-Mannose 28. Tyrosine Arylamidase 29. Citrate (Sodium) 30. β-N-Acetyl-Galactose aminidase 31. L-Histidine assimilation 32. Ellman 33. D-Cellobiose 34. γ-Glutamyl transferase 35. β-Xylosidase 36. Urease 37. Malonate 38. α-Glactosidase 39. Coumarate 40. L-Lactate assimilation 41. β-Galactosidase 42. Fermentation/Glucose 43. β-Alanine Arylamidase pNA 44. D-Sorbitol 45. 5-Keto-D-Gluconate 46. Phosphate 47. β- Glucoronidase.

Test no	Test name	Reaction	Test no	Test name	Reaction
1	APPA	-	25	IARL	-
2	H2S	-	26	dGLU	+
3	BGLU	-	27	dMNE	+
4	PROA	-	28	TyRA	+
5	SAC	-	29	CIT	-
6	ILATK	+	30	NAGA	-
7	GLYA	-	31	IHISA	-
8	O129R	+	32	ELLM	+
9	ADO	-	33	DCEL	-
10	BNAG	-	34	GGT	-
11	dMAL	+	35	BXYL	-
12	LIP	-	36	URE	-
13	DTAG	-	37	MNT	-
14	AGLU	-	38	AGAL	+
15	ODC	+	39	CMT	+
16	GGAA	-	40	ILATA	-
17	PYRA	-	41	BGAL	+
18	AGLTP	-	42	OFF	+
19	dMAN	+	43	BALAP	-
20	PLE	-	44	dSOR	+
21	dTRE	+	45	5KG	-
22	SUCT	+	46	PHOS	-
23	LDC	+	47	BGUR	+
24	IMLTA	-		Probability	95-99%

was isolated and identified from 10 specimens at a rate of 11.11% by conventional methods and VITEK 2 technique biochemical confirmation. Antibiogram analysis of all isolates showed 3 strains (30%) to be MRSA with the following profile: Benzylpenicillin MIC ≥ 0.5 R; Ampicillin MIC ≥ 0.4 R; Oxacillin MIC ≥ 4 R; Gentamicin MIC ≤ 0.5 S; Ciprofloxacin MIC ≤ 0.5 S; levofloxacin MIC ≤ 0.12 S; Moxifloxacin MIC ≤ 0.25 S; Erythromycin MIC 2 IR; Clindamycin MIC 0.5 S; Quinupristin/Dalfopristin MIC 1 S; Linezolid MIC 2 S; Vancomycin MIC 2 S; Tetracycline MIC 2 S; Tigecyclin MIC ≤ 0.12 S; Nitrofurantoin MIC 64 IR; Rifampicin MIC 1 S; Trimethoprim/Sulfa MIC ≥ 320 R.

Rest of isolates showed R or IR to ciprofloxacin; all isolates were sensitive to vancomycin.

B. Enterobacteriaceae: From all specimens, *E. coli* was isolated and identified from 5 specimens at a rate of 5.56% by conventional methods and VITEK 2 technique biochemical confirmation (Table 1). An isolate is considered to be *E. coli* if it gives positive reactions in BGAL, dGLU, OFF, dMAL, dMAN, dMNE, TyRA, dSOR, dTRE, ILATK, SUCT, AGAL, PHOS, ODC, LDC, CMT, BGUR, O129R, ELLM and negative in APPA, H2S, URE. Probability to be *E. coli* was from 95% to 99% in all tested strains and confidence was excellent identification. On SMA, all strains were sorbitol positive indicating absence of *E. coli* O157 strains presumptively.

Employing standard culture and biochemical confirmation methods for the detection of *Salmonella spp.* showed no *Salmonella spp.* in all of the examined food samples.

Antibiogram analysis of all isolates showed 2 strains (40%) to be ESBL positive with the following profile:

ESBL MIC: Pos; Ampicillin: MIC ≤ 2 S; Amoxicillin MIC ≤ 2 S; Piperacillin MIC ≤ 4 S; Cefotaxime MIC ≤ 4 S; Ceftazidime MIC ≤ 1 S; Cefepime MIC ≤ 1 S; Ertapenem MIC ≤ 0.5 S; Imipenem MIC ≤ 0.25 S; Meropenem MIC ≤ 0.25 S; Amikacin MIC ≤ 2 S; Gentamicin MIC ≤ 1 S; Ciprofloxacin MIC ≥ 0.4 R; Norofloxacine MIC ≤ 0.5 S; Fosfomycin MIC ≤ 16 S; Nitrofurantoin MIC ≤ 16 S; Trimethoprim/Sulfa MIC ≤ 20 S.

Antibiogram profile of 3 strains (60%) identified as ESBL negative:

ESBL MIC: Neg; Ampicillin: MIC ≤ 2 S; Amoxicillin MIC ≤ 2 S; Piperacillin MIC ≤ 4 S; Cefotaxime MIC ≤ 4 S; Ceftazidime MIC ≤ 1 S; Cefepime MIC ≤ 1 S; Ertapenem MIC ≤ 0.5 S; Imipenem MIC ≤ 0.25 S; Meropenem MIC ≤ 0.25 S; Amikacin MIC ≤ 2 S; Gentamicin MIC ≤ 1 S; Ciprofloxacin MIC ≤ 0.25 S; Norofloxacine MIC ≤ 0.5 S; Fosfomycin MIC ≤ 16 S; Nitrofurantoin MIC ≤ 16 S; Trimethoprim/Sulfa MIC ≤ 20 S.

Molecular analysis

It indicated that 4 strains belonged to STEC family and one strain was *Shigella flexneri* (Table 3). All strains were *E. coli* non - O157 and strain O157: H7 was not identified from the isolates.

DISCUSSION

The main objective of present study, was to identify *Staph. aureus*, *E. coli* and *Salmonella spp.* from ready-to-eat food as potential pathogens and reservoir of antibiotic resistance for man in the study area. The source of food contamination with *Staph. aureus* (11.11%) from samples investigated, in the present study, could be from minced meat, storage conditions of meat or the food handlers. This is in agreement with another study that detected *Staph. aureus* in ready-to-eat food [10]. Food handlers may be important source of contamination as 27.7% *Staph. aureus* carriage on the hands of asymptomatic food handlers was reported with 50% *Staph. aureus* carriers.

In the present study, *Staph. aureus* was isolated at a rate of 11.11%

Table 2: Anti-microbial agents used for antimicrobial susceptibility testing of *Staph. aureus* and *E. coli* isolates from ready-to-eat meat sandwiches Al-Ahsa province, KSA.

Serial	*Staph. aureus*	*E. coli*	Serial	*Staph. aureus*	*E. coli*
1	Benzyl penicillin	ESBL	10	Quinupristin/Dalfopristin	Meropenem
2	Ampicillin	Ampicillin	11	Linezolid	Amikacin
3	Oxacillin	Amoxicillin	12	Vancomycin	Gentamicin
4	Gentamicin	Piperacillin	13	Tetracycline	Ciprofloxacin
5	Ciprofloxacin	Cefotaxime	14	Tigecyclin	Norofloxacine
6	levofloxacin	Ceftazidime	15	Nitrofurantoin	Fosfomycin
7	Moxifloxacin	Cefepime	16	Rifampicin	Nitrofurantoin
8	Erythromycin	Ertapenem	17	Trimethoprim/ Sulfamethoxazole	Trimethoprim/ Sulfamethoxazole
9	Clindamycin	Imipenem			

Table 3: Comparison of identification by partial 16S rRNA gene sequence analysis and by VITEK 2 technique of *E. coli* isolates from ready-to-eat meat sandwiches in Al-Ahsa province, KSA.

Isolate No.*	Strain Designation	Base Pairs Examined	Bit Score	Homology	Percent	VITEK 2 Identification	Reference
FP 19	*E. coli* O25b:H4-ST131 str. EC958	1491	2723	1484/1489	99%	*E. coli*	emb|HG941718.1|
FP 40	*E. coli* O145:H28 str. RM13514	1493	2724	1477/1478	99%	*E. coli*	gb|CP006027.1|
FP 63	*E. coli* O103:H2 str. 12009	1509	2715	1478/1481	99%	*E. coli*	dbj|AP010958.1|
FP 71	*Shigella flexneri* FBD002	1494	2736	1485/1487	99%	*E. coli*	gb|EU009187.1|
FP 78	*E. coli* O145:H28 str. RM12581	1479	2719	1474/1475	99%	*E. coli*	gb|CP007136.1|

of which 30% was identified to be MRSA from ready-to-eat fast food for the first time in the study area. Studies on meat contamination with pathogenic bacteria or foodborne infection are meager in KSA. Reports of pathogens in food were limited to investigation of food poisoning cases that sought medical care in hospitals. Recently, a study to determine the prevalence of pathogens in domestic refrigerators in Jeddah, KSA showed contamination with *E. coli*, *Salmonella spp.*, *Campylobacter spp.* and *Listeria spp.* [37]. Another study [38], reported presence of highly resistant *E. coli* strains in meat samples from outlets in Taif [39] reported a high incidence of *E. coli*, *Salmonella spp.* and *Staph. aureus* in chicken meat from Al-Ahsa markets. MRSA has been reported in KSA since the 1990s, but still there are few studies on this strain compared to other parts of the world [40]. The above-mentioned studies indicated that MRSA is an important cause of nosocomial infection with high prevalence and quickly increasing in two tertiary-care centres in Jeddah [41-43]. In ready-to-eat food, the infection risk is high because they are not subjected to further cooking at high temperatures. Further surveys are needed on *Staph. aureus* fast-food hygiene issues, in the study area, to collect data on raw material for preparation of meals, storage conditions, methods of service and carriage rate among food-handlers.

Antimicrobial sensitivity test, in the present study, showed MRSA strains to be sensitive to ciprofloxacin while non-MRSA were resistant to ciprofloxacin. However, all strains were sensitive to vancomycin which is the drug of choice for treatment of MRSA strains.

In the present study, on SMA, all *E. coli* strains were sorbitol positive and by the VITEK 2 technique all tested isolates were sorbitol positive (Table 1). Sorbitol fermentation as a sole confirmatory test for *E. coli* O157 strains may be questionable [44]. *E. coli* O157 strains are β-glucoronidase and sorbitol negative. β-glucoronidase appears to be a confirmed characteristic to differentiate between *E. coli* O157 and non-O157 strains. Hence, *E. coli* O157 was not biochemically confirmed among all tested strains in the present study. Due to the importance of STEC as foodborne infection and public health concern, it is important to obtain characteristics of prevailing STEC isolates in the region.

Efficacy of conventional phenotypic identification and molecular techniques was compared in the findings of the present study. By molecular analysis, one strain was identified as ST131 serotype O25b: H4, two as serotype O145:H28 and one as serotype O103:H2, all were confirmed as *E. coli* by VITEK 2 technique. However, one strain was identified as *Shig. flexneri* by molecular technique but identified as *E. coli* by VITEK 2 method. This is considered as misidentification by conventional methods since partial 16S rRNA gene sequencing draws and compiles reference sequences from GenBank, it contains a wide spectrum of comparative sequences for different species. Thus it provides greater microbial diversity data and increases the ability to accurately identify microorganisms. The bit-score provides a better rule-of-thumb for detecting homology in partial 16S rRNA gene sequencing. However, combination of two techniques is useful as currently there is no gold standard for STEC isolation and characterization [12].

By conventional and molecular techniques, *E. coli* O157 was not confirmed in the present study. Other workers isolated *E. coli* O157 at a rate of 4% from meat samples obtained from different slaughterhouses in Riyadh area, KSA [18] plus serotypes O111, O174: H2 and O22: H8. This is in disagreement with the findings of the present study which can be attributed to the difference in specimen types and techniques. In USA, it was reported that sero-types O26, O45, O103, O111, O121, and O145 (big six) are emerging important foodborne infectious agents [45]. This stresses importance of the findings of the present study where two O145:H28 and one O103:H2 sero-types were confirmed.

In the European Union, STEC serotype O157 was the most reported serogroup at rate of 42% in 2015, however, other serotypes and non-typable STEC strains were increasingly reported [46]. In the present study, sandwich samples were prepared from bovine meat which is considered by Hancock et al., [47] to be a major source of foodborne STEC infections in humans. A summary of published reports indicated that the prevalence rates of *E. coli* O157 ranged from 0.3 to 19.7% in feedlots and from 0.7 to 27.3% on pasture with regard to beef cattle and that corresponding prevalence rates of non-O157 STEC were 4.6 to 55.9% and 4.7 to 44.8%, respectively [48]. In USA, a multistate food poisoning outbreak due to hamburgers distributed by a restaurant chain was reported pointing to the efficiency of undercooked ground beef in transmitting STEC infection [49].

Antimicrobial susceptibility, in the present study, showed 40% of *E. coli* isolates to be ESBL positive which is higher than what reported from some European countries [50]. The discrepancy in rates may be attributed to misuse of antibiotics in the study area. Further it has been shown that ESBL producers are susceptible to imipenem but resistant to ciprofloxacin.

CONCLUSION

From ready-to-eat food in Al-Ahsa Province, KSA, *Staph. aureus* MRSA were resistant to benzyl penicillin, ampicillin, oxacillin, erythromycin, nitrofurantoin and trimethoprim/sulfamethoxazole. Non-MRSA were resistant to ciprofloxacin and all susceptible to vancomycin. Using molecular methods, *E. coli* and *Shig. flexneri* were confirmed from food with ESBL positive strains susceptible to imipenem but resistant to ciprofloxacin.

AUTHOR CONTRIBUTIONS

Dr Al-Humam designed the study, did lab analysis of samples and bacteriological investigation. He analysed data, interpreted the results and wrote up the final manuscript.

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